Q-205

Investigation of stress response in the metal reducing organism *Geobacter metallireducens*

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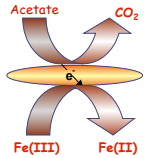
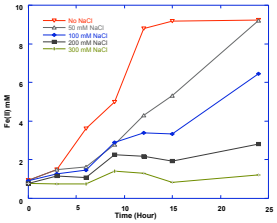
Abstract

In an effort to investigate effects of environmental stressors on bacteria that are found to co-exist in several DOE-contaminated sites, we studied the effect of salt stress on *Geobacter metallireducens* strain GS15. A comparison of these results with salt stress in *Desulfovibrio vulgaris* and *Shewanella oneidensis* will provide a better understanding of bioremediation by these bacteria under hypersaline conditions. Strain GS15 was grown in the defined LS4D medium at 30~C containing 10mM acetate and 10mM Fe-NTA. In these growth conditions, the minimum inhibitory concentration of NaCl on this organism was determined to be 100mM. To examine salt stress response of GS15 in detail, 100mM NaCl was added to a midlog phase culture and cells were harvested after 1, 2 and 4 hours for different analyses including Fe(III) reduction, transcriptomics and phospholipids fatty acid analysis (PLFA). In stressed cultures, after 4 hours of exposure to salt, only 7mM of Fe(II) was reduced compared to more than 9mM in non-stressed controls. Transcriptomic analysis revealed that after 4 hrs, the most up-regulated genes observed were those that encode heat shock proteins, such as Hsp90, Hsp 20, GrpE, DnaK, and DnaJ. These heat shock proteins perform protein repair functions which can be in demand during stress. Also up-regulated were genes encoding proteins in the large and small subunits of the ribosome. The most down-regulated genes included those encoding the, sodium ion symporter, some genes of cytochrome C family and flagellar proteins (flagellin, FlaG, and FlgJ). It was also observed that several proteins involved in the process of chemotaxis like CheA, CheR, CheY, CheX along with several histidine kinases and response regulators were down-regulated. PLFA analysis of stressed cells was performed and compared to non-stressed cells, showing small shifts in relative levels of saturated and unsaturated fatty acids in response to salt stress. The results of this study suggests that presence of 100mM NaCl causes some perturbation in the cell machinery of strain GS15 and that cells are able to overcome this stress without fatal consequences in response to stress.

Introduction



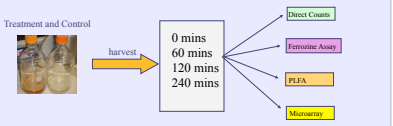
Our experiments were conducted with *Geobacter metallireducens* strain GS15 grown on modified LS4D medium with 5mM acetate as the sole Carbon and energy source and 10mM Fe(III)-NTA as the sole electron acceptor.

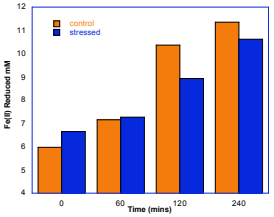


To determine the Minimal Inhibitory Concentration (MIC) of NaCl on strain GS15, a 10% inoculum from a culture in mid-log phase was used to inoculate media amended with 50, 100, 200, 300 mM NaCl and no NaCl control. Optical density readings could not be used to track cell growth due to interference from Fe(III) particles (media turns from orange rust color to clear).

Schematic representation of the experimental design:

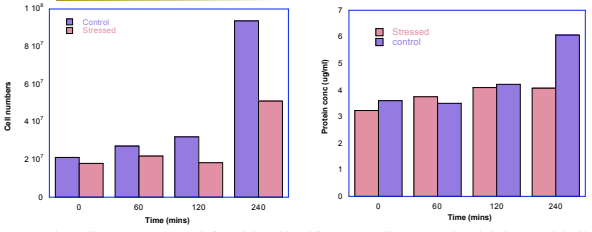
Cultures were grown to mid-log phase.
3 culture bottles exposed to stress.
3 culture bottles not exposed to stress (control).
All culture bottles sampled at 0, 60, 120, 240 minutes after stress for several analyses





Cell growth was tracked as a function of the amount of Fe(III) reduced to Fe(II). After T120mins of adding the stressor, Fe(III) reduction in the bottles were visibly slower than the controls. Fe(II) concentration was determined using the Ferrozine Assay. Cells of GS15 stressed with NaCl reduced Fe(III) to Fe(II) much more slowly and to a lesser extent than non-stressed cells serving as control.

Results



Direct cell counts were measured after staining with acridine orange. Cell growth was impeded when amended with 100mM NaCl. Similar results were seen when the whole cell protein was analyzed.

Phospholipid Fatty Acid Analysis

Phospholipid composition of *Geobacter metallireducens* strain GS 15 in LS4D at 30°C. n = 54

Phospholipid	*Fraction*	Std Deviation
14:0	0.099	0.022
i15:0	0.114	0.013
16:1w7c	0.426	0.083
16:1w5c	0.026	0.012
16:0	0.202	0.039
10Me16:0	0.033	0.051
18:1w9c	0.009	0.015
18:1w7c	0.013	0.007
18:0	0.047	0.071
br18:1	0.031	0.055

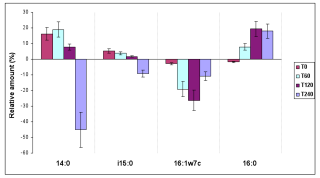
14:0 = 14 carbon saturated (straight chain)

i15:0 = 15 carbon, terminally branched

16:1w7c = 16 carbon, with one unsaturation.

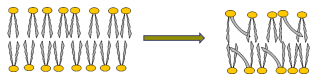
16:0 = 16 carbon, saturated (straight chain)

Relative lipid amount in stressed cells as related to control cells. Positive value indicates enrichment of lipid in stressed cells. Each bar is average of 6 replicates.



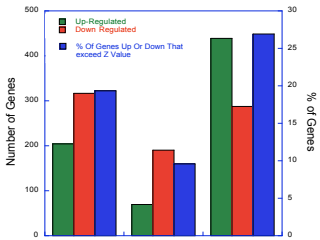
The lipid composition of the stressed cells at 60 minutes, 120 minutes and 240 min were compared to the lipid composition at 0 minutes. Only the following four major lipids (which represent 90% of the lipids in the samples) were used in the analysis. During NaCl stress, decrease of 14:0, some decrease of branched and unsaturated lipids i15:0 and 16:1w7c and an increase in 16:0 fraction observed.

During salt stress, on average, the cell walls or the population are getting thicker (increase in 16:0 with reduction in 14:0) and less branched. The reduction in branched lipids (i15:0) and unsaturated lipids (16:1w7c) allows for closer packing of the lipid bilayer tails and increases membrane fluidity.

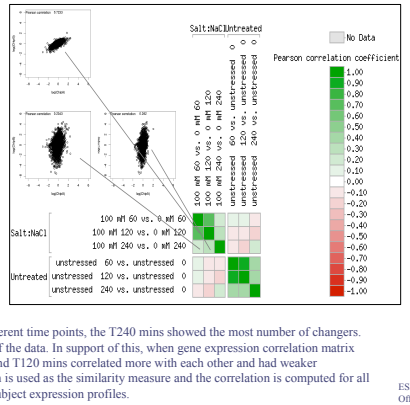


Schematic demonstrating effect of lipid tail composition on geometry

Genome wide microarray analysis



To determine if the change in gene expression (stress vs. control) was statistically significant, a Z value of 2 was used as a cut-off. The Z value was calculated using the following equation:
$Z = (\log_2(\text{treatment/control}))/(0.25+\sum\text{variance})^{1/2}$



When we plotted the gene changes with 'z' >2 for data at different time points, the T240 mins showed the most number of changers. So we focused on this particular sample for further analysis of the data. In support of this, when gene expression correlation matrix was plotted, it was evident from the heatmap that T60 mins and T120 mins correlated more with each other and had weaker correlation with T240 mins. The centered Pearson correlation is used as the similarity measure and the correlation is computed for all pairs of experiments which have data in both the query and subject expression profiles.

Clusters of Orthologous Groups of proteins (COGs) at T240 mins were delineated by comparing protein sequences encoded in complete genomes

COG Function Categories	Total	Up	Down
Amino acid transport and metabolism	157	42	7
Carbohydrate transport and metabolism	94	16	11
Cell cycle control, cell div, chromosome partitioning	25	4	2
Cell motility	119	0	52
Cell wall/membrane/envelope biogenesis	190	32	5
Chromatin structure and dynamics	1	0	0
Coenzyme transport and metabolism	129	31	6
Defense mechanisms	34	2	0
Energy production and conversion	279	29	34
Function unknown	195	32	22
General function prediction only	289	29	31
Inorganic ion transport and metabolism	108	9	14
Intracellular trafficking, secretion, vesicular transport	89	16	17
Lipid transport and metabolism	134	22	5
Nucleotide transport and metabolism	58	26	3
Posttranslational modifn, protein turnover, chaperones	108	21	17
Replication, recombination and repair	162	12	2
Secondary metabolites biosynth, transport, catabolism	54	9	4
Signal transduction mechanisms	191	5	43
Transcription	124	18	10
Translation, ribosomal structure and biogenesis	159	84	2

Some Significant Up-regulated genes coded for these category of proteins:

- **Coenzyme metabolism:** Synthesis of biotin, s-adenosylmethionine,siroheme, thiamine
- **Amino acid biosynthesis and transport:** Especially pyruvate, glutamate, aspartate family
- **Ribosomal protein synthesis**: L9, L25, L6, S2 , S18 and others involved in ribosome structure and translation.
- **Fatty acid and phospholipid biosynthesis and transport:**
- **Protein folding and stabilization:** DnaK, GroEL, GrpE. These are also seen upregulated under heat shock conditions and salt stress in other bacteria.
- **Peptide and Protein secretion and trafficking:** YajC, SecF, SecD
- **Protein degradation**: ClpP, ClpX

Some Significant Down-regulated genes coded for these category of proteins:

- **Inorganic ion transport and metabolism:** K+ potassium transporter, Bacterioferritin
- **Sodium/substrate symporter:** Solute transport across cytoplasmic membranes
- **Energy production and conversion**: NADH dehydrogenase, cytochrome b/b6 like complex.
- **Cell motility:** FliS, FliN, FlaG, PilT and several others.
- **Signal transduction**: CheW, CheY, CheD and others along with associated response regulators, histidine kinases.

We applied the Fischer Exact Test to compute the probability that more down- or up- changing genes could be observed. Using this test and a significance threshold of p-value < 0.0001 (x10-4), three COG categories showed highly significant gene expression changes.
Cell motility: 5.19021E-23,
Signal transduction mechanisms: 1.19874E-13,
Translation, ribosomal structure and biogenesis:1.36396E-17

Conclusions

- The response of *G metallireducens* strain GS15 to salt stress was studied. The salt MIC was found to be 100mM. Cell numbers, amount of Fe(III) reduced, total cell protein all decreased when cells were stressed with 100mM salt.
- PLFA analyses suggests that *G metallireducens* strain GS15 increases the hydrophobicity of its membrane by increasing the fraction of long chain saturated lipids in the membrane during salt stress. This is to counterbalance the increased ionic strength of its environment.
- Microarray analysis of cells under salt stress indicates that the most number of gene changers are at 240mins.
- Further, while genes responsible for protein repair, degradation and folding were highly up-regulated to combat salt stress, those for key cellular processes like replication, transcription and translation were not affected.
- Genes for cell motility, signal transduction, ion transport were down-regulated but those for synthesis of cell membrane, coenzymes and amino acids were up-regulated to allow for adjustments to the cell membrane for survival under higher salinity.

Acknowledgement

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